Decreased RAGE expression in peripheral blood mononuclear cells of patients with rheumatoid arthritis

S. Drinda[1], S. Franke[1], T. Eidner[1], C. Schmidt[2], C. Rüster[1], T. Bondeva[1], G. Hein[1], G. Wolf[1]

[1]Department of Internal Medicine III, and [2]Department of Internal Medicine II, Division of Gastroenterology, Friedrich-Schiller-University, Jena, Germany.

Abstract

Objective

Interactions between the multiligand receptor for advanced glycation end products (RAGE) and its proinflammatory ligands (AGEs, S100/calgranulins, HMBG1, Mac-1) may contribute to inflammatory responses playing a key role in the pathogenesis of chronic inflammatory diseases such as in rheumatoid arthritis (RA). Peripheral blood mononuclear cells (PBMCs) participate in the development of chronic inflammatory diseases. This study investigated expression of the RAGE variants endogenous secretory RAGE (esRAGE), N-truncated RAGE (NtRAGE) and complete RAGE (cRAGE: encoding full-length RAGE, esRAGE and NtRAGE) in PBMCs of patients with RA in comparison to healthy control subjects (controls) and to patients with Crohn's disease (CD) as another chronic inflammatory disease.

Methods

The cRAGE, esRAGE and NtRAGE mRNA expression levels of PBMCs from controls, RA and CD patients were measured by real-time PCR. The RAGE protein expression was determined by Western blot analysis and the esRAGE plasma levels by ELISA.

Results

PBMCs of RA patients showed significantly decreased mRNA expression for cRAGE (46%), esRAGE (54.0%) and NtRAGE (52%) in comparison to healthy controls (100%). For CD patients, also a down-regulation but to a lower extent was found (cRAGE: 79%; esRAGE: 76%; NtRAGE: 69%). Related to controls, RA PBMCs showed a significantly reduced protein expression of full-length RAGE (53%) as well as significantly decreased esRAGE plasma concentrations (70%).

Conclusion

The down-regulation of RAGE isoforms in RA PBMCs may contribute to reduced intracellular responses mediated by the cell-standing receptor as well as to a lowered capability of trapping inflammatory ligands by circulating esRAGE.

Key words

Rheumatoid arthritis, Crohn's disease, peripheral blood mononuclear cells, RAGE expression.

Stefan Drinda, MD*
Sybille Franke, PhD*
Thorsten Eidner, MD
Carsten Schmidt, MD, PhD
Christiane Rüster, MD
Tzvetanka Bondeva, PhD
Gert Hein, MD, Professor
Gunter Wolf, MD, Professor

*Both authors contributed equally to this study.

Please address correspondence and reprint requests to:
Sybille Franke, PhD,
Department of Internal Medicine III,
Friedrich-Schiller-University,
Erlanger Allee 101,
D-07740 Jena, Germany.
E-mail: sybille.franke@med.uni-jena.de

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by the key features of autoimmunity, chronic inflammation and joint destruction. Among others, the degree of systemic inflammation has been identified as a reliable predictor of disease progression in early RA, suggesting that persistent systemic inflammation drives the disease to a tissue-destructive course (1).

Recently, the receptor for advanced glycation end products (AGEs), named RAGE, appears to play an important role in inflammatory processes (2). RAGE is a multiligand member of the immunoglobulin superfamily which can be activated by a variety of proinflammatory ligands including AGEs, S100 proteins (*e.g.* S100A12, S100B), high-mobility group box 1 protein (HMGB1) and Mac-1 (3-5). RAGE-ligand interaction results in a rapid and sustained cellular activation of NF-κB, accompanied by increased expression of the receptor itself (6). For RA fibroblast-like synoviocytes, it was demonstrated recently that RAGE activation contributes to the characteristic, pathological invasive behaviour of these cells (7).

In addition to the cell-standing full-length receptor, different RAGE mRNA splice variants have been identified encoding for truncated proteins with different biological properties (8, 9). The major isoforms of RAGE are on the one hand the membrane-standing molecules full-length RAGE, dominant-negative RAGE (dnRAGE) and N-truncated RAGE (NtRAGE). The NtRAGE is not able to bind ligands. On the other hand, the soluble RAGE forms (sRAGE) circulating in the blood consists of the splice variant endogenous secretory RAGE (esRAGE) lacking the transmembrane domain of the receptor as well as of proteolytically cleaved forms shed into the circulation by action of metalloproteinases. Both, esRAGE and sRAGE have previously been demonstrated to act as decoys by binding ligands and to counteract the activation of RAGE-mediated intracellular pathways via NF-κB (10, 11).

AGEs (such as N^{ε}-carboxymethyllysine or pentosidine), HMGB1, S100A12 and Mac-1 as RAGE ligands were found to be elevated in serum, synovial tissue or synovial fluid samples in RA (5, 12-15). Consistent with the concept that enhanced accumulation of these ligands contributes to up-regulation of the receptor, increased expression of cell surface RAGE has been demonstrated in human RA for endothelial cells and synovial macrophages (16).

To get a better insight into these processes, we investigated whether there is a changed expression of RAGE in peripheral blood mononuclear cells (PBMCs) of RA patients. PBMCs are implicated in the primary autoimmune and inflammatory responses of RA triggering the disease to tissue destruction and represent an established target to investigate gene expression changes in RA (17).

We also studied RAGE isoforms in the PBMCs of patients with RA in comparison to healthy control subjects and to patients with Crohn's disease as another chronic inflammatory disease.

Materials and methods
Patients
The study included 22 patients with rheumatoid arthritis (RA), 13 patients with Crohn's disease (CD) and 24 healthy control subjects (controls). The patients were recruited consecutively in the outpatients department of the Departments of Internal Medicine II and III (University Hospital Jena).

All RA patients fulfilled the 1987 revised American College of Rheumatology criteria for the diagnosis of RA (18). As parameter of disease activity the Disease Activity Score 28 (DAS28) was used. The DAS28 considers 28 tender and swollen joint counts, erythrocyte sedimentation rate (ESR), general health and patient assessment of disease activity. The score can be used to objectively evaluate a patient's response to treatment (19, 20).

Diagnosis of Crohn's disease has been established by standard criteria, combining clinical presentation, endoscopic and histologic examination, as well as radiologic evaluation in individual cases. Disease activity has been determined by means of the Crohn's disease activity index (CDAI), that is containing

clinical data (stool frequency, abdominal pain, general well-being), presence of extraintestinal manifestations and/or abdominal mass, use of anti-diarrhoic medication, hematocrit and body weight (21, 22).

The apparently healthy volunteers recruited from the staff of the Department of Internal Medicine III were without clinical signs of any diseases and had normal laboratory values on a standard screening panel.

Informed consent for study participation was given by all patients and healthy subjects. The study was approved by the local ethics committee. Anticoagulated whole blood samples (EDTA-treated) were taken between 08:00 and 10:00 hours. PBMCs and plasma were separated immediately after venipuncture.

Reagents

The following reagents were used: Ficoll-Paque Plus (Amersham Biosciences, Freiburg, Germany) for PBMC separation; RNA lysis buffer, RNeasy Mini Kit, RNase-Free DNase Set (Qiagen, Hilden, Germany) for RNA extraction, Reverse Transcription System (Promega, Madison, USA) for cDNA synthesis, FastStart DNA Masterplus SYBR Green I-Kit (Roche Diagnostics, Mannheim, Germany) for real-time PCR, Complete Lysis-M buffer for protein extraction (Roche Diagnostics, Mannheim, Germany), BCA protein assay kit for quantification of total protein (Pierce, Rockford, USA). For immunostaining: primary antibodies anti-RAGE (C-20, Santa Cruz Biotech, Santa Cruz, USA; SP6366P, Acris Antibodies, Hiddenhausen, Germany) and anti-β-Actin (Sigma, St. Louis, USA),

HRP-conjugated secondary antibodies (KPL, Gaithersburg, USA), Western Lightning Chemiluminescence Reagent Plus (Perkin Elmer LAS, Boston, USA). Enzyme-linked immunosorbent assay (ELISA) for determination of esRAGE plasma levels (B-Bridge International, Sunnyvale, USA).

Preparation of peripheral blood mononuclear cells (PBMCs)

20 ml whole blood were diluted with 30 ml phosphate buffered saline (PBS) and loaded carefully in equal parts onto two Ficoll-Paque Plus gradients (20 ml each). After centrifugation at 400 g for 40 min at 18-20°C the PBMCs were aspirated and washed three times in PBS at pH 7.4 and 4°C. After counting the cells were divided, pelleted and transferred into appropriate volumes of RNA lysis buffer (Qiagen) or protein lysis buffer (Roche Diagnostics), respectively.

For characterization, the cells were analyzed by FACS using forward and side scatter measurement (FSC, SSC) which allows to distinguish between lymphocytes, monocytes and granulocytes according to their physical properties. After gating, the number of respective cells was given in the FSC/SSC plots. The isolated cell fraction consisted of greater than 90% of PBMCs (lymphocytes and monocytes). The remaining <10% of cells constituted of granulocytes, erythrocytes and cell debris.

Total RNA extraction and reverse transcription

Total cellular RNA was extracted using the RNeasy Mini Kit (Qiagen) supplementing the standard protocol by DNase

digeston with the corresponding RNase-Free DNase Set. RNA yield and purity was determined using a NanoDrop ND-1000 spectrophotometer. Complementary DNA (cDNA) was synthesized from 1 μg of total RNA with the Reverse Transcription System (Promega).

Real-time PCR

Real-time PCR was performed with the Realplex Mastercycler instrument (Eppendorf AG, Hamburg, Germany). For preparation of the Master Mix, the FastStart DNA Masterplus SYBR Green I-Kit (Roche Diagnostics) was used. Together with the specific primers, the Master Mix was added to cDNA solutions. The cDNA samples were amplified according to the manufacturer's instructions. Non-template controls were included to ensure specifity. The sequences of the chosen primers and the cycler conditions are given in the Table I. Each sample was measured in duplicate.

The real-time PCR data were plotted as the intensity of fluorescence signal vs cycle number. The cycle threshold (Ct) value is defined as the cycle number at which the fluorescence signal crosses the threshold fluorescence. Initially, the reference and target amplification efficiencies were evaluated analyzing the Ct values of template dilutions within a 10000-fold range and calculated from the slopes of the semi-log regression lines according to the equation $E=10^{(-1/slope)}$ (23). The relations between target amplification (cRAGE, esRAGE, NtRAGE) and reference amplification (GAPDH) in each individual sample were defined as: $\Delta Ct_{cRAGE}=Ct_{cRAGE}-Ct_{GAPDH}$, $\Delta Ct_{esRAGE}=Ct_{esRAGE}-Ct_{GAPDH}$, $\Delta Ct_{NtRAGE}=Ct_{NtRAGE}-Ct_{GAPDH}$.

Table I. DNA sequences of the sense and antisense primers for real-time PCR analysis and cycler conditions.

Gene identity	Accession number	Primer sequences	Annealing temperature (°C)	Number of cycles	Product size (bp)
GAPDH	J02642	5'-CAATGACCCCTTCATTGACC-3' (sense) 5'-TGGACTCCACGACGTACTCA-3' (antisense)	59	30	197
RAGE	AB036432	5'-GGAAAGGAGACCAAGTCCAA-3' (sense) 5'-CATCCAAGTGCCAGCTAAGA-3' (antisense)	59	30	166
esRAGE	AB061668	5'-TGTGTGGCCACCCATTCCAG-3' (sense) 5'-GCTATCTTCTGCTTCCCTGAC-3' (antisense)	59	35	129
NtRAGE	AB061669	5'-TGCCTTTCAAGGTCCCTCAC-3' (sense) 5'-GGCCTGTGTTCAGTTTCCAT-3' (antisense)	62	35	204

Western blots

For Western blotting, isolated PBMCs were lysed in complete Lysis-M buffer (Roche Diagnostics) and the protein concentration was determined using the BCA protein assay kit (Pierce). After incubating the protein extracts in sodium dodecyl sulphate (SDS) sample buffer at 100°C for 5 minutes, aliquots of 30 µg protein/lane were electrophoresed in a 12% acrylamide SDS-polyacrylamide gel. Proteins were transferred to a polyvinylidene fluoride (PVDF) membrane using a semidry transfer cell (Bio-Rad Laboratories, Hercules, USA). Nonspecific binding sites were blocked for 1 h with 5% bovine serum albumin in Tris-buffered saline (Tris, pH 7.4) and 0.1% Tween-20 followed by overnight incubation at 4°C in primary antibodies to RAGE (polyclonal goat, Cruz Biotech, directed against a peptide mapping the C-terminus; polyclonal rabbit, Acris, raised against a peptide corresponding to the N-terminus of human RAGE) or to β-actin (monoclonal mouse, 1:4000, Sigma). The membrane was then washed four times for 5 minutes in Tris buffer containing 0.1% Tween-20, and incubated with the corresponding horseradish peroxidase-linked secondary antibody (KPL). Detection of peroxidase was performed with an enhanced chemiluminescent reagent (Perkin Elmer LAS). For imaging and digitization the LAS-3000 imaging system (Fujifilm Life Science, Düsseldorf, Germany) was used.

The densities of RAGE bands recognized by both antibodies at ~50 kDa were measured using the Phoretix-1D-Software (Phoretix 1D Advanced, Nonlinear Dynamics, Newcastle, UK) and normalized for the respective densities of β-actin bands as loading controls.

ELISA for esRAGE

Plasma levels of esRAGE protein were determined in RA and control samples in duplicates by ELISA according to the manufacturer's instructions (B-Bridge International, Sunnyvale, USA).

Statistical analysis

All data are presented as the means ± SEM (standard error of the mean). Statistical significance between different groups was first tested with the non-parametric Kruskal-Wallis test. Individual groups were subsequently tested using the Mann-Whitney U-test. For correlation analysis the Spearman's correlation test was used. A p-value of <0.05 was considered significant.

Results

The clinical characteristics of patients are summarized in Table II.

RA patients were significantly older then controls and CD patients and were more consequently treated with DMARDs alone or in combination with prednisolone and biologicals. Means of disease duration, CrP, ESR, haematocrit and leucocytes of both patient groups were not significantly different. The RA group had in mean moderate disease activity (as indicated by the DAS28) and consisted of good therapy-responders, moderate responders and non-responders. The CD patients were in remission or experienced a low disease activity of Crohn's disease.

An overview of the studied RAGE variants is shown in Figure 1. As an example, the semi-log Ct vs cDNA dilution plot with the corresponding regression line for cRAGE amplification is given in Figure 2. The data confirmed that the amplification efficiencies of all amplicons were approximately equal.

All PBMC samples had detectable levels of cRAGE, esRAGE and NtRAGE mRNA.

In none of the investigated groups significant correlations between the individual mRNA expression levels of the measured RAGE isoforms and the subjects age as well as the disease duration were found. In addition, the disease activity score (DAS28) of the RA patients as well as the Crohn's disease activity index (CDAI) did not correlate with the respective expression levels (*e.g.* DAS28-cRAGE expression: r=-0.302, p=0.239, n.s.; CDAI-cRAGE expression: r=-0.517, p=0.154, n.s.).

The PBMC mRNA expression of all RAGE isoforms was lower in both patient groups than in healthy controls (Fig. 3). CD patients showed a mean reduction to 79% for cRAGE, 76% for esRAGE and 70% for NtRAGE suggesting a down-regulation of approximately 20 to 30%. For RA patients significant
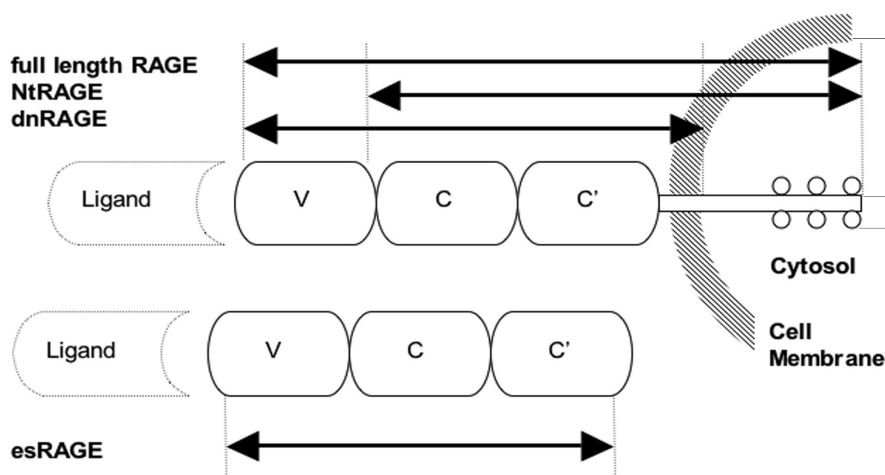
Table II. Patients characteristics.

(mean ± SD)	Rheumatoid arthritis	Crohn's disease	p-value (RA *vs.* CD)	Control subjects
n	22	13		24
Sex (female/male)	18 / 4	8/5		21 / 3
Age (years)	55 ± 15	33 ± 15	p<0.001	37 ± 14
Range	(31-74)	(18-66)		(19-60)
Disease duration (years)	11 ± 7	8 ± 5	n.s.[3]	0
Disease activity	4.4 ± 1.4 (DAS28)	77.4 ± 55.6 (CDAI)		
CrP (mg/l)	14 ± 16	17 ± 30	n.s.	not done
ESR (mm/h)	23 ± 17	16 ± 12	n.s.	not done
Haematocrit (%)	42 ± 4	40 ± 3	n.s.	not done
Leucocytes (Gpt/l)	8 ± 2	8 ± 4	n.s.	not done
Drug treatments:				
No DMARDs[4]	2	5		24
Methotrexate (MTX)	8	1		0
Leflunomide (LEF)	1	0		0
Azathioprine (AZA)	0	7		0
MTX + infliximab[5]	2	0		0
MTX + adalimumab[5]	3	0		0
MTX + eternacept[5]	3	0		0
LEF + infliximab[5]	1	0		0
LEF + adalimumab[5]	2	0		0
Prednisolone	15	4		0
	(daily dose 2-7 mg)	(daily dose 3-10 mg)		

[1] C-reactive protein, [2] Erythrocyte sedimentation rate, [3] not significant, [4] Disease modifying anti-rheumatic drugs: azathioprine, leflunomide, methotrexate; [5] Biologicals: TNF-α blocking substances adalimumab, eternacept, infliximab.

Fig. 1. Structure of the receptor for advanced glycation endproducts (RAGE) and its major splice variants. The molecule consists of 3 extracellular domains (V-C-C'). The V domain interacts with ligands. The RAGE isoforms are the full-length RAGE (binds ligands and mediate activation of intracellular signal transduction pathways), the N-truncated RAGE (Nt-RAGE, not able to bind ligands), the dominant-negative RAGE (DN-RAGE, lacking the cytosolic tail and binds extracellular ligands without intrinsic effects) and endogenous-secretory RAGE (esRAGE; binds extracellular ligands and prevents epitop recognition by full-length receptor).
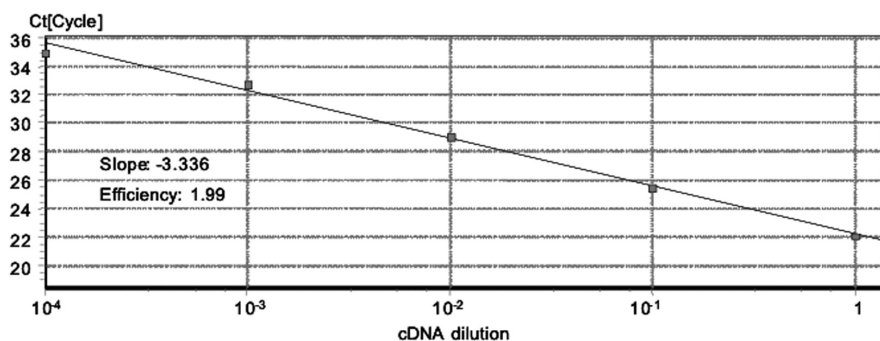

Fig. 2. Efficiency of cRAGE amplification by real-time PCR.
Serial dilutions of cDNA (10^{-4} to 1) were amplified using primers specific for cRAGE. The slope of the semi-log regression line of the Ct *vs.* cDNA dilution plot was -3.336 corresponding to an amplification efficiency of 1.99.
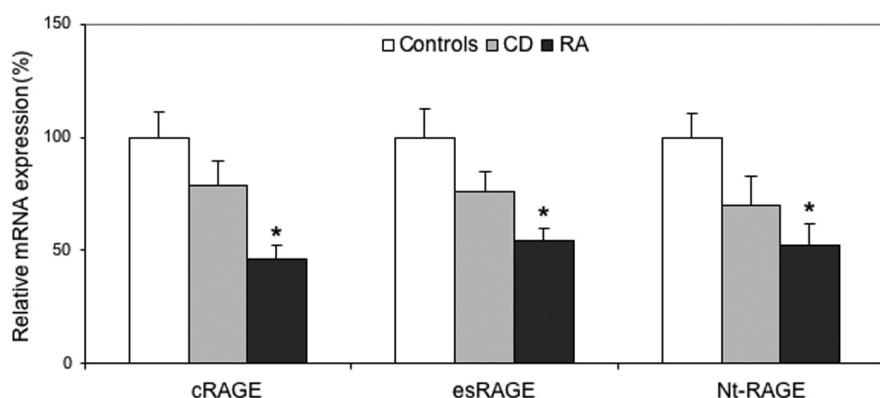

Fig. 3. cRAGE, esRAGE and Nt-RAGE mRNA expression analyzed by real-time RT-PCR.
In comparison to healthy controls CD PBMCs showed a non-significantly down-regulated expression of all investigated isoforms, whereas for PBMCs of RA patients a significant reduction was found (*$p \leq 0.002$ *vs.* controls).

reductions in means to 46% for cRAGE, 54% for esRAGE and 52% for NtRAGE were found. These data indicate that the RAGE expression of RA PBMCs was approximately 50% lower than in healthy PBMCs.

Although the RA patients received different therapies, the PBMCs of the resulting subgroups showed similar expression levels for cRAGE, esRAGE and NtRAGE. These suggest that the RAGE expression was not specifically influenced by these treatments. In contrast, the receptor expression of PBMCs from CD patients receiving azathioprine were similar to healthy subjects, whereas in patients without basic therapy down-regulated levels were found. For example, the cRAGE mRNA expression levels of PBMCs from RA and CD patients corresponding to different drug therapies in comparison to healthy subjects are given in Table III.

Prednisolone was given in both patient groups in combination with DMARDs or DMARDs and biologicals. Thus, a specific effect of prednisolone on receptor expression could not be evaluated. However, even though statistically not significant we found the receptor expression levels of RA and CD patients receiving prednisolone lower than those without prednisolone treatment (Table III). Furthermore, a subgroup of RA patients treated with methotrexate and prednisolone (n=5) showed stronger reduced levels than RA patients on methotrexate monotherapy (n=3) (cRAGE: 39±10 *vs*. 57±17%; esRAGE: 39±11 *vs*. 76±12%; NtRAGE: 40±15 *vs*. 66±28%). The respective findings for CD patients receiving azathioprine alone (n=4) or in combination with prednisolone (n=3) were similar.

To examine the impact of lowered RAGE mRNA expression on the protein levels, we measured in PBMC lysates (8 RA and 8 control lysates; Western blot analysis) the expression of the full-length form corresponding to a band at ~50 kDa (24) as well as the esRAGE concentrations in RA and control plasma samples (ELISA). In comparison to healthy controls, the expression of the full-length receptor was significantly decreased in RA PBMCs to 53% (Fig. 4) and the esRAGE plasma levels of RA patients to 70%, respectively (Fig. 5).

Discussion

Monocytes and lymphocytes are involved in the pathogenesis of RA,

Table III. cRAGE mRNA expression measured in peripheral blood mononuclear cells of patients with RA and CD corresponding to different drug treatments in comparison to healthy controls.

| means±SEM	Relative cRAGE mRNA expression (% of controls)			
	RA	p vs controls	CD	p vs. controls
No basic therapy	53 ± 27 (n=2)	n.s.	54 ± 20 (n=5)	n.s.
Methotrexate	45 ± 7 (n=8)	<0.001	66 (n=1)	n.s.
Methotrexate + biologicals	48 ± 12 (n=8)	0.003		
Leflunomide	45 (n=1)	n.s.		
Leflunomide + biologicals	46 ± 18 (n=3)	0.004		
Azathioprine			104 ± 10 (n=7)	n.s.
Prednisolone	44 ± 8 (n=15)	<0.001	59 ± 20 (n=4)	n.s
No prednisolone	55 ± 7 (n=7)	<0.001	88 ± 12 (n=9)	n.s

n.s. not significant.
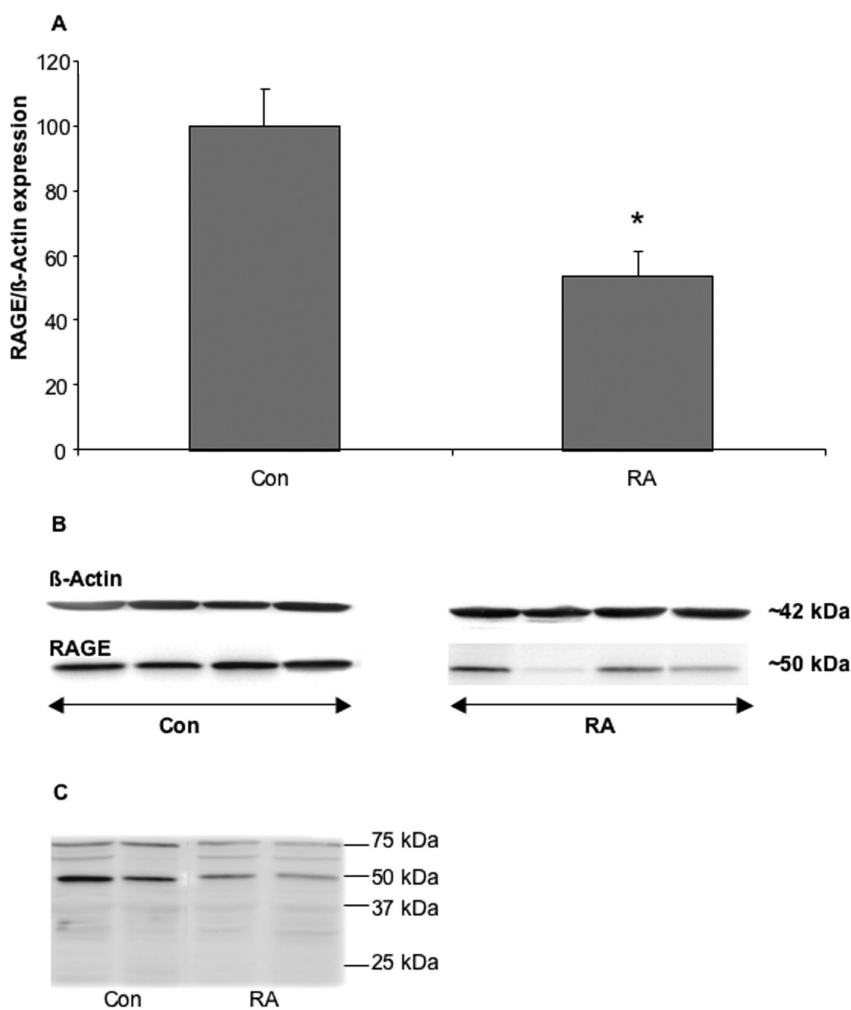
Fig. 4. RAGE protein expression determined by Western blot analysis of whole PBMC lysates. (**A**) In comparison to healthy controls the expression of full-length RAGE was significantly decreased in PBMC lysates of RA patients. The RAGE protein expression of RA PBMCs was in mean 47% lower than in the respective control cells (*p<0.05). (**B**) For example, four representative blots of RA and control samples are given. (**C**) Additionally, the whole blots of two RA and two control samples are shown demonstrating that the antibody raised against a peptide corresponding to the N-terminus of human RAGE did not detect truncated RAGE forms.

tissue specific cells like synovial macrophages or fibroblasts, PBMC samples can be obtained more easily. In the present study, PBMCs were used to investigate the mRNA expression of complete RAGE and of its splice variants esRAGE and NtRAGE in patients with RA in comparison to normal volunteers and patients with Crohn's disease, another chronic inflammatory disease too.

We could clearly demonstrate that the mRNA expression of cell-bound as well as of endogeneous secretory RAGE was lower in RA than in healthy subjects. These data were confirmed at the protein level, indicating a decreased de-novo synthesis of the full-length receptor. It can be suggested this fact is a likely reason for decreased plasma and synovial fluid levels of circulating RAGE (sRAGE consisting of esRAGE and proteolytically cleaved RAGE) in RA as reported by Pullerits *et al.* (26). In difference to our own investigation, in this reported study no discrimination was made between different circulating RAGE forms and the cellular sources of the measured sRAGE were not delineated. In CD patients a similar result but to a lesser degree was found. The RAGE expression was approximately 20 to 30% reduced as compared to healthy subjects.

In none of the investigated groups the individual RAGE expression levels were correlated with the subjects age. This may indicate that the down-regulation found for RA was not caused by the higher mean age of the patients in comparison to those of controls. The lowered mRNA expression of cRAGE (encoding full-length RAGE, esRAGE and NtRAGE) in PBMCs of patients without basic therapy as well as of patients receiving methotrexate or biologicals suggests independence of the observed down-regulation by these drugs. In contrast, administration of azathioprine seems to counteract this process resulting in similar expression levels like in healthy subjects. However, most of the patients were on combined therapy. The groups of RA patients classified RA according to treatment are to small to produce statistically significant results. Therefore, the specific influence of in-
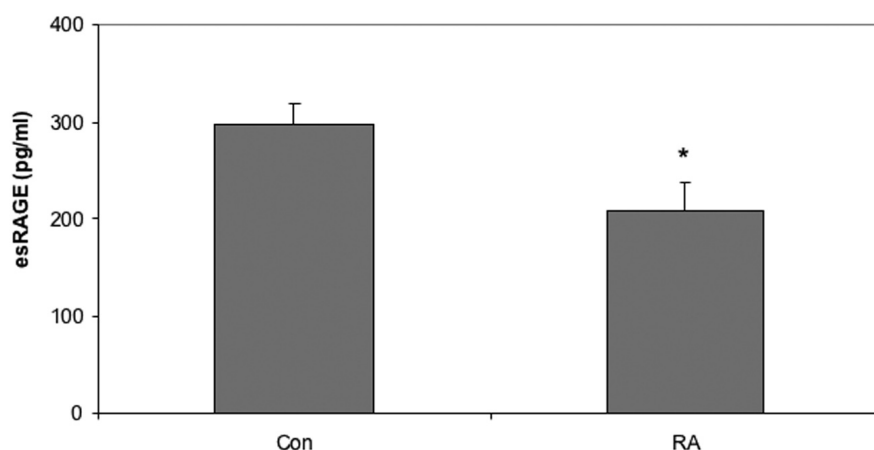
especially in autoimmune and inflammatory processes. Recent studies demonstrate that gene expression analysis on these cells also in peripheral blood can provide insight into the disease pathogenesis (17, 25). In contrast to

Fig. 5. esRAGE plasma levels of RA patients and healthy controls measured by ELISA. As compared to healthy controls RA patients had significantly decreased esRAGE plasma levels with a mean reduction of 30% (*$p<0.05$).

dividual drugs could not be evaluated. The changes in RAGE expression of RA PBMCs might be the result of complex processes: firstly, long-lasting contacts with the large variety of inflammatory factors (*e.g.* proinflammatory cytokines) occurring during the ongoing disease may alter the expression profile of the cells. The fact, that RAGE-ligand interactions lead to NF-κB-mediated cell activation and enhanced production of proinflammatory cytokines is well accepted, but how in turn prolonged higher levels of inflammatory agents affect the RAGE expression in vivo is still unknown. Secondly, changes in the extracellular and intracellular RAGE ligand profile caused by the disease itself and/or by immunosuppressive therapy may modulate the RAGE expression too. However, whether the changed RAGE expression might be a consequence of the disease or of different disease-modifying antiinflammatory therapies remains an open question and needs further investigation.

PBMCs of course do not exactly represent the situation within the inflamed synovial membranes of RA. RA is a systemic disease however and lowered sRAGE protein levels are reported for RA synovial fluid too (26). In this regard it would be of high interest to know, how synovial mononuclear cells present in the microenvironment of the inflamed joint express RAGE. During acute inflammation these cells are exposed to higher levels of inflammatory cytokines than blood cells.

Recently it was introduced that RAGE is implicated in the innate immune network as a pattern recognition receptor for damage-associated molecular pattern molecules (DAMPs) confirming its complex action mechanism. HMGB1 and S-100 proteins as ligands of membrane-bound and soluble RAGE represent important danger signals that mediate inflammatory responses through the receptor (27, 28).

Until now, the exact mechanisms by which circulating RAGE regulates inflammatory responses is incompletely understood. In the literature, controversial data of soluble RAGE levels are given. In diabetic patients increased as well as decreased plasma levels were found (11, 29, 30). Because of the observed association with its ligand S100A12 and other proinflammatory factors, soluble RAGE is suggested to be a biomarker of inflammation exerting a protective role against the development of diabetic complications. Both soluble forms, esRAGE and proteolytically cleaved RAGE, were shown to act as decoys binding inflammatory RAGE ligands (11). For RA decreased levels of soluble RAGE are discussed to increase the propensity towards inflammation (26). This is consistent with our data showing a decrease of de-novo synthesis of esRAGE in the peripheral blood of RA patients. Moreover, our findings demonstrate that these goes by with a lowered expression of cell-bound RAGE too, indicating a dependently regulated expression of the

receptor isoforms in PBMCs. Strongly reduced RAGE isoforms at the mRNA and at the protein levels are reported for lung carcinomas, but in contrast an independent regulation was suggested (31, 32).

In summary, from our results it can be proposed that reduced RAGE levels of RA PBMCs contribute to diminished intracellular responses mediated by the full-length receptor as well as to a lowered capability for binding inflammatory ligands by circulating esRAGE. These suggest that both, the proinflammatory as well as antiinflammatory action of RAGE is suppressed in PBMCs of RA patients.

Whether down-regulation of the RAGE mRNA expression in peripheral blood cells is a genetically fixed predisposing factor, a consequence of the disease itself or influenced by disease-modifying antiinflammatory and immunosuppressive therapy needs to be further elucidated. The study presented here is afflicted with some limitations. In this regard, further studies should evaluate the influence of anti-rheumatic drugs like glucocorticoids, methotrexate and TNF-α-blocking substances on gene expression and protein levels of the RAGE variants *in vitro* and *in vivo*.

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